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Appl. No. 09/463,082
Amdt. Dated November 12, 2003 OR TO BE DETERMINED
Reply to Office Action Of July 14, 2003

REMARKS/ARGUMENTS

In the Office Action of July 14, 2003, claims 31, 33-35, 61, 64, 68, 71 and 72 were rejected under 35 USC § 103(a) as unpatentable over International Patent Application No. WO93/15768 (Watson *et al.*) in view of U.S. Patent No. 5,640,705 (Koruga) and in view of U.S. Patent No. 5,217,705 (Reno *et al.*). In addition, claim 72 was rejected under 35 USC § 112 as being indefinite.

The amendments and remarks as presented here are believed to place the case in condition for allowance. Accordingly, entry of these amendments, reconsideration and allowance is respectfully requested. It is believed that a one month extension is required with this submission. Therefore, a petition for a one month extension and fee of $ 110.00 are provided. With this response claims 31, 33-35, 61, 64, 68 and 71 are pending herein.

Correction of the Record

In the amendment of January 27, 2003, Applicants characterized the "soot" particles ("structured aggregates of carbon") disclosed in the Burch *et al.* reference as "hydrophobic" and "thermodynamically unstable in an aqueous environment." Upon further analysis and review of the scientific literature, Applicants now note that these generalizations relating to the physical and chemical properties of carbonaceous particulate are overly simplified. The hydrophobic or hydrophilic nature of the interaction of carbonaceous particles depends on the precise chemical form of the carbon surface layers which are exposed to an aqueous medium. Graphitic carbon surfaces, for example, have been demonstrated to form a contact angle with water at 300 K of 86° degrees, which indicates that these surfaces readily associate with the water and are actually hydrophilic (See, Exhibit A, "Water-Carbon Interactions: Potential Energy Calibration Using Experimental Data," T. Werder, J.H. Walther, R.L. Jaffe and P. Koumoutsakos). In contrast, fullerenic carbon surfaces typically require derivatization, such as the addition of solubilizing

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groups, to provide for significant association with water and appreciable hydrophilic behavior. Applicant's oversimplification of the physical and chemical properties of carbonaceous particles was inadvertent and without any deceptive intent.

The Amendments

Amendment of the specification is requested to include a cross reference to related applications. The amended specification is now consistent with the priority information set forth in International Application PCT/AU98/00582 of which the present application is a National Stage Application filed under 35 U.S.C. § 371. The requested correction corrects an obvious clerical error and does not add new matter.

Amendment of claim 31 is requested to more particularly point out and distinctly claim the present invention. Specifically, the method of claim 31 now specifies that the outer surface of the particles "comprises graphitic carbon." Support for this amendment is provided by the description of exemplary diagnostic carbonaceous particles comprising a "detectable marker . . . encased in about 2 to 20 layers of graphitic carbon, more preferably about 2 to 10 layers of graphitic carbon." In addition, support for this amendment is provided by the discussion of how the diagnostic particles of the present invention differ from fullerene derivate particles on page 3, lines 19 to 28. The requested amendment does not introduce any new matter.

Rejections under 35 U.S.C. § 103

Claims 31, 33, 34, 61 and 71 have been rejected under Section 103(a) as being unpatentable over International Patent Application No. WO93/15768 (Watson et al.) in view of U.S. Patent No. 5,640,705 (Koruga) and in view of U.S. Patent No. 5,217,705 (Reno et al.). In support of this rejection, the Office Action characterizes Watson et al. as disclosing "the use of fullerenes in diagnostic and/or therapeutic agents" and Koruga as teaching that fullerenes "can be

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formed as concentric multi-layer carbon cages." With respect to the combined teaching of Watson and Koruga, the Examiner concludes that:

> [i]t would have been obvious to one of ordinary skill in the art at the time the invention was made to teach the fullerenes of Watson as comprising two or more carbon layers, as taught by Koruga, because of the expectation of achieving a product, wherein the contents of the fullerene are more fully trapped and not as susceptible to leaking out of the carbon skeleton matrix, thereby forming a more sustained-release formulation.

Further, the Examiner characterizes Reno *et al.* as teaching a "method of diagnosing blood clots using fibrin-binding proteins" and concludes that:

> [i]t would have been obvious to one of ordinary skill in the art at the time the invention was made to teach fibrin-binding proteins, as taught by Reno *et al.*, as the proteins of Watson *et al.* because Watson *et al.* teach their agents for targeting the blood pool and because of the expectation of achieving a stable contrast agent that is able to locate harmful fibrin blood clots.

Applicant has amended the rejected claims to more clearly specify the claimed invention and requests reconsideration and withdrawal of the rejections in light of the following arguments.

First, amended claims 31, 33, 34, 61 and 71 are not anticipated by the combination of references cited because the diagnostic particles used in the claimed methods and the materials disclosed in Watson *et al.* and Koruga have materially different chemical structures. Although the diagnostic particles of the present invention and the materials disclosed in the cited references are comprised of carbon, the particles used in the present methods comprise graphitic carbon. In contrast, the disclosure in Watson *et al.* and Kurga is limited to carbon in the form of fullerenes comprising carbon lattices having bond angles and overall geometries that differ materially from the six carbon repeated, aromatic ring structure of graphite. To clarify this distinction, claim 31 has been amended to recite "the outer surface of said particles comprises graphitic carbon." The express limitation of "graphitic carbon" in the amended claims

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distinguishes the composition of the present diagnostic particles from the fullerenic materials of Watson *et al.* and Kurga. Claims 31, 33, 34, 61 and 71 are not rendered obvious by the cited references because the combination of Watson *et al.*, Koruga and Reno *et al.* fails to teach or suggest all the limitations in the amended claims and the missing claim limitations are well outside the grasped of the typical artisan at the time of invention. *See, e.g.,* In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). It is therefore submitted that no *prima facie* case of nonobviousness has been made out with respect to this rejection, and withdrawal thereof is respectfully requested.

Second, the differences in the chemical structures of the diagnostic particles in the amended claims and the materials described in Watson *et al.* and Kurga give rise to substantially different chemical and physical properties, which further functionally distinguish the present diagnostic particles from the fullerenic materials of Watson *et al.* and Kurga.

Graphitic carbon surfaces make a contact angle with water of less than 90°, which indicates that the surface of the particles of the present invention are naturally hydrophilic in nature (see, Exhibit A, wherein the contact angle of water on graphite is reported to be 86° at 300 K). Indeed, graphitic carbon undergoes strong association with water allowing for the formation of stable and long lasting colloidal suspensions (see, Exhibit A, wherein a bond energy for the association between graphite and water is calculated to be -7.3 KJ mol^{-1}). This property of the particles of the present invention is important because it allows for their effective use in aqueous colloidal suspensions. In contrast, fullerenes are highly insoluble in aqueous media, typically requiring labor intensive and difficult chemical functionalization of their outer surfaces to provide for effective dissolution. Thus, unlike the fullerenes of Watson *et al.* and Kurga, the diagnostic particles of the present invention **do not** require derivatization or chemical modification to make a stable aqueous dispersion. Rather it is the hydrophilic nature of the graphite structure itself that provides for this functionality.

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In addition, the reactivity of the unmodified graphitic carbon surfaces of the diagnostic particles of the present invention is characterized by high affinity and selective binding to fibrin. This property of the particles of the present invention is important because it allows for their use for the *in vivo* detection of fibrin. In contrast, the combination of the teachings of Watson *et al.*, Koruga and Reno *et al.* relied upon by the Examiner requires complex derivatization synthesis reactions to provide effective coupling of fibrin-binding proteins to fullerene surfaces. Therefore, unlike the combination proposed by the Examiner, the diagnostic particles of the present invention **do not** require derivatization or chemical modification to provide selective binding to fibrin. Rather, this important functional attribute is provided by the unmodified graphite structure itself.

Third, the combined teachings of Watson *et al.*, Koruga and Reno *et al.* do not enable the synthesis of compositions comprising fullerenic materials having surfaces modified to include fibrin binding proteins and solubilizing groups. Fullerenes are very stable compounds and derivatization of their surfaces involves highly specific chemical addition reactions. There is no teaching in the combination of references relating to the manner or chemical pathway involved in coupling fibrin binding proteins to the highly inert surfaces of fullerenes. Indeed, the Reno *et al.* reference does not describe or suggest diagnostic markers comprising carbonaceous materials. Second, there is no teaching in the combination of references relating to the synthesis of fullerenes having a plurality of different affinity groups, such as solubilizing groups and fibrin binding proteins. References which do not enable one skilled in the art to construct and practice a claimed invention are not properly cited as prior art. *See, e.g.*, In re Hoeksema, 158 U.S.P.Q. 596, 600-01 (CCPA 1968). It is therefore submitted that no *prima facie* case of nonobviousness has been made out with respect to this rejection, and withdrawal thereof is respectfully requested.

Fourth, the Examiner does not provide any credible motivation to combine the teachings of teachings of Watson *et al.*, Koruga and Reno *et al.* to arrive at carbonaceous diagnostic materials exhibiting selective binding to fibrin and capable of forming a stable aqueous

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dispersion. The Examiner suggests that it would have been obvious to one of ordinary skill in the art to combine the teaching of Watson *et al.* with that of Koruga to arrive at a diagnostic material comprising two or more carbon layers because of the expectation of achieving a product "wherein the contents of the fullerene are more fully trapped and not as susceptible to leaking out of the carbon skeleton matrix, thereby forming a more sustained-release formulation". Fullerenes are extremely "leak proof", so much so that they can contain helium and hydrogen elements which are some of the most diffusive species known. Therefore, the proposed motivation to combine the teachings of Watson *et al.* and Koruga is not credible because the pores in fullerenes are on the scale of atomic dimensions and, thus, are too small to allow for effective release of enclosed detectable markers.

In addition, the Examiner asserts the "expectation of achieving a stable contrast agent that is able to locate harmful fibrin blood clots" as a motivation to combine the fullerenes disclosed in Watson *et al.* with the fibrin binding proteins described in Reno *et al.* The present application relates to the *in vivo* detection of fibrin using the discrete particles of the invention dispersed in a suitable carrier, diluent, excipient or adjuvant, which can accumulate marker at vascular sites of fibrin deposition. In contrast, the methods of Watson *et al.* provide contrast agents which travel in the blood stream and do not localize. Therefore, the motivation of providing a stable contrast agent relied upon by the Examiner does not lead to the asserted combination of the teachings in Watson *et al.* and Reno *et al.*

Fifth, the combined teachings of Watson *et al.*, Koruga and Reno *et al.* does not provide a reasonable expectation of successfully preparing carbonaceous materials having surfaces modified to include fibrin binding proteins and solubilizing groups and using such materials for *in vivo* fibrin detection. Particularly, a person of ordinary skill in the art would not expect that fibrin binding proteins can be chemically coupled to inert carbon surfaces, such as fullerene surfaces, without affecting protein structure and ability to selectively bind to fibrin. Proteins are very labile and are known to readily undergo conformational changes when their chemical

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environment is changed. In addition, a person of ordinary skill in the art would not expect that fibrin binding proteins and solubilizing groups can be sequentially coupled to inert carbon surfaces, such as fullerene surfaces, without disrupting their respective functioning (e.g. providing selective binding to fibrin and appreciable solubility). References which do not provide a reasonable expectation of successfully practicing the invention as claimed are not properly cited as prior art. *See, e.g.*, Amgen, Inc. v. Chugai Pharmaceutical Co., 927 F.2d 1200, 18 USPQ2d 1016, 1022 – 23 (Fed. Cir.), *cert denied*, 502 U.S. 856 (1991). It is therefore submitted that no *prima facie* case of nonobviousness has been made out with respect to this rejection, and withdrawal thereof is respectfully requested.

<u>Rejections under 112</u>

Claim 72 has been rejected under Section 112 as being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicant regards as the invention. Specifically, the term "lyophilic" in claim 72 is allegedly vague and indefinite. To expedite prosecution and without acquiescing to this rejection, claim 72 has been cancelled.

CONCLUSION

In view of the foregoing, this case is considered to be in condition for allowance and passage to issuance is respectfully requested. It is believed that a one month extension is required with this submission. Therefore, a petition for a one month extension and fee of $ 110.00 are provided. If this is incorrect, please deduct the appropriate fee for this submission and any extension of time required from Deposit Account No. 07-1969.

Respectfully submitted,

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Water-Carbon Interactions: Potential Energy Calibration Using Experimental Data

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ABSTRACT

The water-graphite interaction is characterized by molecular dynamics simulations of water droplets on a graphite surface. The binding energy of a single water molecule on graphite is found to be directly proportional to the well depth (ε_{CO}) in the Lennard Jones potential for the pairwise carbon-oxygen interaction and inversely proportional to the droplet contact angle. ε_{CO} is adjusted to enable the simulation results to match the literature value for the contact angle at 300 K (86°). This results in a binding energy of -7.3 kJ/mol for a single water molecule on graphite and a value of 0.4389 kJ/mol for ε_{CO}.

Keywords: carbon nanotubes, water, molecular dynamics, contact angle, potential energy.

1 INTRODUCTION

Functionalized carbon nanotubes (CNT) are being studied for use as nanoscale biosensors (Nguyen 2002) and water or proton channels (Hummer 2001, Werder 2001, Noon 2002). Several molecular dynamics (MD) studies of water in carbon nanotubes have recently been performed to gain further insight into the latter, but in these MD studies a wide range of intermolecular potentials for water and water-carbon interactions was employed (see Werder 2002 for details). In general, experimental data are not available for calibrating the strength of the water-carbon interaction, so it is difficult to assess the accuracy of these potentials. In the present study, we use MD simulations to compute the static contact angle for nanometer-size water droplets on graphite and compare the results with available experimental data (Fowkes 1938 as cited in Adamson and Gast 1997). This leads to a new approach for determination of the interaction potential parameters.

2 RESULTS AND DISCUSSION

We use the SPC/E water model (Berendsen 1987) and compare different strength water-carbon interactions for the case of a 2000-molecule water droplet on a graphite surface (a typical case is shown in Figure 1). The graphite is modeled by two 119x118 Å graphene sheets fixed in a staggered configuration 3.4 Å apart. The water-carbon interaction is modeled by a pairwise Lennard-Jones potential, $E_{CO} = \sum 4\varepsilon_{CO}[(\sigma_{CO}/R_i)^{12} - (\sigma_{CO}/R_i)^6]$, where the index i runs over all pairs of C and O atoms and R_i is the distance between atoms in the ith pair. The well depth and position of the minimum in the potential are given by ε_{CO} and $(2^{1/6})\sigma_{CO}$, respectively.

Figure 1: Snapshot of water droplet on graphite from MD simulation at 300 K using the SPC/E water model, and a carbon-oxygen Lennard-Jones potential with parameters $\varepsilon = 0.4389$kJ/mol and $\sigma = 3.19$ Å.

For each set of water-carbon parameters, we calculate the static contact angle (θ) of the droplet (see Figure 2) and the binding energy (ΔE) of a single water molecule on the graphite surface. In so doing, we demonstrate that the contact angle, which is commonly used to characterize the wettability of a substrate, is sensitive to changes in the carbon-water interaction strength. We also demonstrate that our small-droplet results can be extrapolated to macroscopic droplets, which allows us to use the experimental value for the contact angle of water on
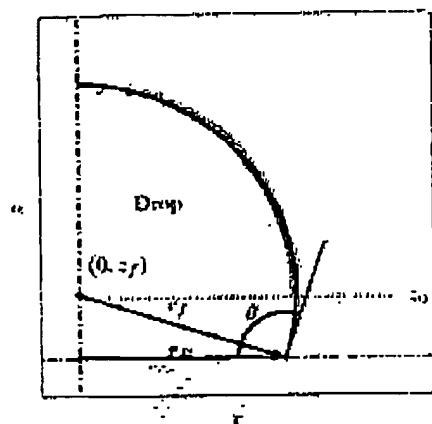
Figure 2: Schematic for calculating the contact angle of a water droplet on graphite from MD simulations. The contact angle is measured by fitting a circle with center (0, z_f) and radius r_f to the points of the equimolar dividing plane (circles) with $z > z_0 = 8$ Å to exclude the near wall region. The droplet has a base radius r_B.

Figure 4: Lennard-Jones well depth parameter ε_{CO} for the water-graphite interaction as a function of ΔE, the water molecule binding energy on graphite.

graphite (86°) to calibrate the water-carbon potential. Using a series of water-carbon potentials that range from hydrophobic ($\theta > 90°$) to complete wetting ($\theta = 0°$) behavior in our MD simulations, we determine the relationship between the binding energy of a single water molecule on graphite and θ for the droplet (Figure 3) and between ΔE and the value of ε_{CO} in the water-carbon potential (Figure 4). The data show complete wetting for $\Delta E < -13.1$ kJ/mol and linear relationships between ΔE and θ for other values and between ΔE and ε_{CO}. Thus, we can

accurately predict the binding energy for a water molecule on graphite to yield a specific θ and use that ΔE value to determine the optimal value for ε_{CO}. This represents a new method for calibrating molecular dynamics interaction potentials. Our procedure leads to the recommendation of new water-carbon Lennard-Jones parameters $\varepsilon_{CO} = 0.4389$ kJ/mol and $\sigma_{CO} = 3.19$ Å (Werder 2002). These results are summarized in Table 1.
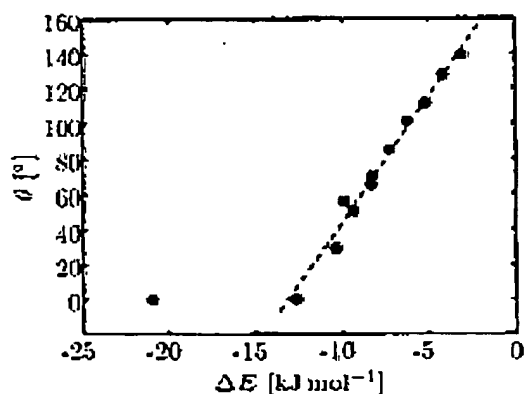

Figure 3: Static contact angle θ of water droplets on graphite as a function of ΔE, the binding energy of a single water molecule on graphite.
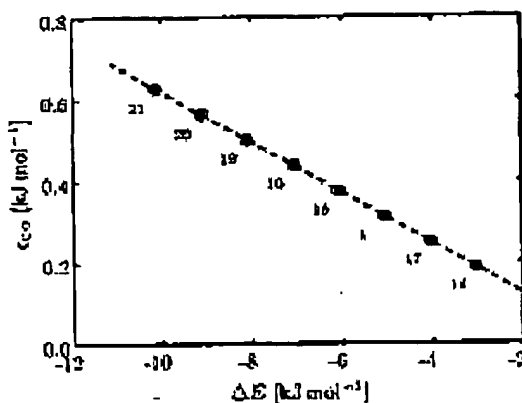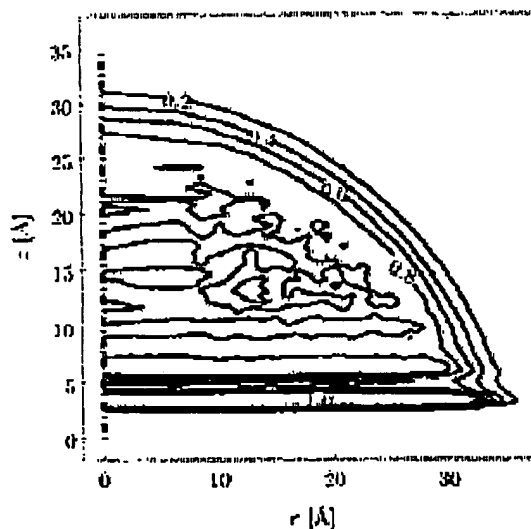

Figure 4: Isochore profile for a water droplet on graphite. The density values for the isochores are 0.2, 0.4, 0.6, 0.8 and 1.0 g/cm³.

We have used this new potential to compute the binding energy of a single water molecule on graphite ($\Delta E = -7.3$ kJ/mol) and the contact angle for a water droplet ($\theta = 85.5°$). We have also characterized the water-graphite/carbon nanotube interface. Figure 5 shows the time-averaged isochore profile for a 2000-water molecule droplet using the new water-carbon parameters. That profile shows layering of the water at the graphite surface with a thin layer of high density ($\rho > 2\rho_{bulk}$) centered 3.2 Å above the surface and second layer ($\rho = 1.5\rho_{bulk}$) centered at a height of 5.5 Å. The droplet assumes bulk water density approximately 10 Å above the surface. The liquid-vapor surface of the droplet where the density drops from the bulk value of 1.0 g/cm3 to nearly zero is about 5 Å thick.

Based on our analysis of the water-carbon interaction strength, we can comment on the results of various studies of graphite or carbon nanotube interactions with water. In particular, the carbon-oxygen potentials employed by Markovic (1999) and Noon (2002) are too strongly attractive. This leads to enhanced hydrophilic behavior and wetting. On the other hand, the potential used by Walther (2001) results in insufficient attraction between the water

and the carbon surfaces leading to enhanced hydrophobic behavior. We are currently repeating some of these simulations using the new water-carbon interaction potential.

REFERENCES

W. Adamson and A. P. Gast, *Physical Chemistry of Surfaces*, 6th ed. (John Wiley & Sons, New York, 1997).

H. J. C. Berendsen, J. R. Grigera and T. P. Straatsma, J. Phys. Chem. 91, 6269 (1987).

F. M. Fowkes, Ph.D. Thesis, University of Chicago, 1938.

M. C. Gordillo and J. Marti, Chem. Phys. Lett. 329, 341 (2000).

G. Hummer et al., Nature 414, 188 (2001).

K. Koga et al., Nature 412, 802 (2001).

N. Markovic et al., Chem. Phys. 247, 413 (1999).

C. V. Nguyen et al., Nano Lett. 2, 1079 (2002).

W. H. Noon et al., Chem. Phys. Lett. 355, 445 (2002).

J. H. Walther et al., J. Phys. Chem. B 105, 9980 (2001).

T. Werder et al., Nano Letters 1, 697 (2001).

T. Werder et al., J. Phys. Chem. B, in press (2002).

Table 1. Summary of Water-Carbon Interaction Models Considered in the Present Study[a]

Study	Water Model	σ_{CO} (Å)	ε_{CO} (kJ/mol)	ΔE (kJ/mol)	θ (°)
Markovic 1999	SPC	3.190	.3910	-12.64	0.0
Gordillo and Marti 2000	SPC-FLX[b]	3.280	.3890	-9.91	55.9
Walther 2001	SPC-FLX	3.190	.3135	-5.19	111.3
Hummer 2001	TIP4P	3.275	.4785	-8.33	48.0
Koga 2001	TIP4P	3.262	.3876	-6.70	
Noon 2002	TIP3P	3.296	.5781	-16.72	0.0
Present Study	SPC/E	3.190	.4389	-7.26	85.5

a. The Markovic, Gordillo and Noon models also included pairwise Lennard-Jones potential terms between carbon atoms and the water hydrogen atoms.

b. SPC-FLX is a flexible water model that allows OH stretch and HOH bending motions.